

EXHIBIT B20

Part 4

	1	2	3	4	5	6	7	8	9	10	11	12
A				Blank 10x		279						
B				Blank		281						
C				Media 10x		297						
D						299						
E				Blank 10x		333						
F						335						
G						351						
H						353						

Re-measure media protein

1-19-18

- 10x diluted samples
- media was 10x as blank
- used 25ul to detect

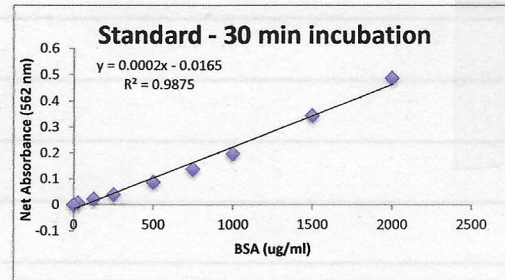
	1	2	3	4	5	6	7	8	9	10	11	12
A							278			335		
B							279			350		
C							281			351		
D							296			353		
E							297			Lysis Buffer		
F							299			Lysis Buffer + talk		
G							332					
H							333					

protein → New control

$$44 \times 200 = \frac{8800}{50} = 176$$

Go to pg 17

Standard ID	Concentration (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
A	2000	0.5869	0.5848	0.5562	0.575967	0.4874
B	1500	0.4457	0.4211	0.3533	0.4334	0.344833
C	1000	0.3213	0.2774	0.2593	0.286	0.197433
D	750	0.2473	0.2199	0.2128	0.226667	0.1381
E	500	0.181	0.1759	0.1713	0.176067	0.0875
F	250	0.1328	0.1252	0.1256	0.127867	0.0393
G	125	0.1105	0.112	0.1116	0.111367	0.0228
H	25	0.1004	0.0941	0.0953	0.0966	0.008033
I	5	0.0845	0.0846	0.0941	0.087733	0
J (BLANK)	0	0.0881	0.0884	0.0892	0.088567	0



	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ul 1	ug/ul 2	ug/ul 3	Average	how much was used	ug used
TOV112D 72 hr unt	278	0.6692	0.6792	0.6667	0.559867	0.6792	0.6667	28.81833	34.785	34.16	34.4725	3.42	117.9
TOV112D 72 hr control	279	0.2121	0.2206	0.2113	0.102767	0.2206	0.2113	5.963333	11.855	11.39	11.6225	14.23	165.4
TOV112D 72 hr 1000 ug/ml Talc	281	0.1866	0.1902	0.189	0.077267	0.1902	0.189	4.688333	10.335	10.275	8.432778	20.21	170.4
Ov90 72 hr unt 72 hr	296	0.3372	0.3405	0.3354	0.227867	0.3405	0.3354	12.21833	17.85	17.595	15.88778	7.94	126.1
Ov90 72 hr control	297	0.3217	0.3231	0.3255	0.212367	0.3231	0.3255	11.44333	16.98	17.1	17.04	8.33	141.9
Ov90 72 hr 1000 ug/ml Talc	299	0.2813	0.282	0.2785	0.171967	0.282	0.2785	9.423333	14.925	14.75	14.8375	10.73	159.2
TOV-121G unt	332	0.4161	0.4393	0.452	0.306767	0.4393	0.452	16.16333	22.79	23.425	23.1075	5.61	129.6
TOV-21G 72 hr control	333	0.3031	0.3039	0.3238	0.193767	0.3039	0.3238	10.51333	16.02	17.015	14.51611	9.70	140.8
TOV-21G 72 hr 1000 ug/ml Talc	335	0.1847	0.1856	0.19	0.075367	0.1856	0.19	4.593333	10.105	10.325	8.341111	27.47	229.1
Normal Ov Epithelial 72 hr unt	350	0.2227	0.2338	0.2367	0.113367	0.2338	0.2367	6.493333	12.515	12.66	12.5875	13.71	172.6
Normal Ov Epithelial 72 hr control	351	0.2287	0.2316	0.2402	0.119367	0.2316	0.2402	6.793333	12.405	12.835	12.62	14.37	181.3
Normal Ov Epithelial 72 hr 1000 ug/ml talc	353	0.2277	0.2391	0.2432	0.118367	0.2391	0.2432	6.743333	12.78	12.985	9.761667	12.94	126.3
Lysis buffer blank		0.1102	0.1083	0.1095	0.109333								
Lysis buffer+talc blank		0.1073	0.1068	0.1059	-0.00203	-0.00253	-0.00343	-0.00267					

Remeasured Media Protein - 10x diluted using 25ul standard

Samples	Raw 1	Raw 2	Raw 3	Corr 1	Corr 2	Corr 3	ug/ul 1	ug/ml 2	ug/ml 3	Avg	xdf	ul media used	actual ug used
279	0.7155	0.7569	0.7571	0.6192	0.6606	0.6608	0.490545	0.528182	0.528364	0.515697	5.15697	92.992174	479.5578
281	0.7067	0.7139	0.7356	0.6104	0.6176	0.6393	0.482545	0.489091	0.508818	0.493485	4.934848	87.280476	430.7159
297	1.1257	1.1418	1.1656	1.0294	1.0455	1.0693	0.863455	0.878091	0.899727	0.880424	8.804242	58.801368	517.7015
299	1.1303	1.1325	1.1842	1.034	1.0362	1.0879	0.867636	0.869636	0.916636	0.884636	8.846364	60.557443	535.7132
333	0.7006	0.7217	0.7401	0.6043	0.6254	0.6438	0.477	0.496182	0.512909	0.495364	4.953636	83.231246	412.2973
335	0.7315	0.7361	0.7684	0.6352	0.6398	0.6721	0.505091	0.509273	0.538636	0.517667	5.176667	89.314713	462.3525
351	0.9217	0.9433	0.9655	0.8254	0.847	0.8692	0.678	0.697636	0.717818	0.697818	6.978182	67.973964	474.3347
353	0.9401	0.9952	1.0083	0.8438	0.8989	0.912	0.694727	0.744818	0.756727	0.732091	7.320909	71.353555	522.3729
Blank-10x media	0.7339	0.7117	0.7024	0.6376	0.7117	0.7024	0.70705						
Blank-PBS	0.0941	0.0998	0.095	0.0963									

Recalculated how much protein was really used and then adjusted CA125 levels pg 18

CA125 - recalc. for SKI abstract

1/12/2018														
MEDIA														
Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation	Corrected per ug protein	Corrected per ug protein	Corrected per ug protein
TOV112D 72 hr control	279	0.104	0.0885	0.0969	0.03735	0.02185	0.03025	5.13076923	2.74615385	4.03846154	4.58461538	0.77237818	0.01069896	0.00572643
TOV112D 72 hr 1000 ug/ml Talc	281	0.0806	0.0742	0.0838	0.01395	0.00755	0.01715	1.53076923	0.54615385	2.02307692	1.77692308	0.34611411	0.00319204	0.00113867
OV90 72 hr control	297	0.1059	0.1046	0.1321	0.03925	0.03795	0.06545	5.42307692	5.22307692	9.45384615	5.32307692	0.14142138	0.01133849	0.01089144
OV90 72 hr 1000 ug/ml Talc	299	0.1062	0.0906	0.1316	0.03955	0.02395	0.06495	5.46923077	3.06923077	9.37692308	4.26923077	1.69705627	0.01140474	0.00640013
TOV-21G 72 hr control	333	0.0724	0.0716	0.0942	0.00575	0.00495	0.02755	2.6923077	0.14615385	3.62307692	0.20769231	0.08702853	0.00056141	0.00030477
TOV-21G 72 hr 1000 ug/ml Talc	335	0.0761	0.077	0.0856	0.00945	0.01035	0.01895	0.83846154	0.97692308	2.3	0.90769231	0.09790709	0.00174841	0.00203713
Normal Ov Epithelial 72 hr control	351	0.1003	0.0943	0.1001	0.03385	0.02765	0.03345	4.56153846	3.63946154	4.53076923	4.54615385	0.02175713	0.00951197	0.00756712
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	353	0.1106	0.092	0.1331	0.04395	0.02535	0.06645	6.14615385	3.28461538	9.60769231	4.71538462	2.02341325	0.01281629	0.00564926
b		0.0661	0.0672		0.06665									0.02003448
1/17/2018														
Lysate														
Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation	Corrected per ug protein	Corrected per ug protein	Corrected per ug protein
TOV112D 72 hr unt	278	0.1998	0.1904	0.171	0.0476	0.0382	6.70769231	5.26153846		5.98461538	1.02235519	0.05099502	0.04462898	0.0508
TOV112D 72 hr control	279	0.1637	0.1749	0.171	0.0807	0.0919	0.088	11.8	13.5230769	12.5230769	13.2230769	0.42426407	0.0713473	0.08176568
TOV112D 72 hr 1000 ug/ml Talc	281	0.1188	0.1175	0.1221	0.0358	0.0345	0.0391	4.89230769	4.69230769	5.4	4.99487179	0.36482413	0.02870627	0.02753275
OV90 72 hr unt 72 hr	296	0.1978	0.1971	0	0.0466	0.0449		6.4	6.29230769		6.34615385	0.07614996	0.05073367	0.04987998
OV90 72 hr control	297	0.146	0.1479	0.1689	0.063	0.0649	0.0859	9.07692308	9.36923077	12.6	9.22307692	0.20689275	0.06394757	0.0660089
OV90 72 hr 1000 ug/ml Talc	299	0.1371	0.13	0.1506	0.0541	0.047	0.0676	7.70769231	6.81538462	9.76461538	7.16153846	0.77237818	0.04841321	0.04155226
TOV-21G unt	332	0.1875	0.179	0.199	0.0353	0.0268	0.0468	4.81538462	3.50769231	6.58461538	4.16153846	0.9246781	0.03714627	0.02705862
TOV-21G 72 hr control	333	0.1374	0.1269	0.1289	0.0544	0.0439	0.0459	7.75384615	6.13846154	6.44615385	6.28230769	0.21757132	0.05506747	0.04359508
TOV-21G 72 hr 1000 ug/ml Talc	335	0.1	0.0869	0.0907	0.017	0.0059	0.0077	2	0.29230769	0.56923077	0.43076923	0.19561419	0.00872896	0.00127573
Normal Ov Epithelial 72 hr unt	350	0.2533	0.2874	0.2328	0.1011	0.1352	0.0806	14.9384615	20.1846154	11.7846154	13.3615385	2.230106	0.08656233	0.11656166
Normal Ov Epithelial 72 hr control	351	0.1491	0.1518	0.1547	0.0661	0.0688	0.0717	9.55384615	9.96923077	10.4153846	9.76153846	0.29372128	0.05266198	0.0549725
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	353	0.1551	0.1362	0.1467	0.0721	0.0532	0.0637	10.4769231	7.56923077	9.18461538	8.83076923	0.91379983	0.07782681	0.05992299
b		0.0868	0.0876	0.0746	0.083									0.07271144

	fold increase	fold increase	fold increase	Average	SD
OV90	1.02745665	0.5765896	1.76156069	1.39450867	0.51908995
TOV 21G	4.03703704	4.7037037	11.0740741	4.37037037	0.47140452
Normal Ov Epi	1.35194585	0.72250423	2.11336717	1.73265651	0.53840618

Media calculations for OV90, 21G, Normal Ov. Epi were used for SKI abstract

Seed 72 hour 1000 $\mu\text{g}/\text{ml}$ tac EOC

SKW-3 and A2780 seeded 4×10^6 cells

TCV-21G seeded 2×10^6 cells

Treat SKW-3 and A2780 today 1/29/18
25ml of media

$$(x)(100 \text{ mg/ml}) = (25 \text{ ml})(1000 \text{ } \mu\text{g}/\text{ml})$$
$$x = 250 \text{ } \mu\text{L}$$

Treat TCV21G 1/30/18

1/31/18 - The presence of 1000 $\mu\text{g}/\text{ml}$ is physically
killing the cells.
- We need to decrease dose.

Treat EOC w/talc

- Needed to lower the dose of talc (in saline) ^{PBS} → 72 hours
- unt, 5, 20, 100 $\mu\text{g/ml}$

- Seeded 1×10^6 cells

ID

60mm dish, 5mL

- ELI unt	356
ELI 5 $\mu\text{g/ml}$	357
ELI 20 $\mu\text{g/ml}$	358
ELI 100 $\mu\text{g/ml}$	359
- SKOV-3 unt	360
5 $\mu\text{g/ml}$	361
20 $\mu\text{g/ml}$	362
100 $\mu\text{g/ml}$	363
- TCV1120 unt	364
5 $\mu\text{g/ml}$	365
20 $\mu\text{g/ml}$	366
100 $\mu\text{g/ml}$	367
- A2780 unt	368
5 $\mu\text{g/ml}$	369
20 $\mu\text{g/ml}$	370
100 $\mu\text{g/ml}$	371
- OVC40 unt	372
5 $\mu\text{g/ml}$	373
20 $\mu\text{g/ml}$	374
100 $\mu\text{g/ml}$	375

FT 33

- No transformation unt	379
5 $\mu\text{g/ml}$	380
20	381
100	382
1000	383
Control 5	377
Control 1000	378

ID

ELI Control-5	379
Control-1000	380
1000 $\mu\text{g/ml}$ talc	381
SKOV-3 Control 5	382
Control 1000	383
1000 $\mu\text{g/ml}$ talc	384
A2780 Control 5	385
Control 1000	386
1000 $\mu\text{g/ml}$ talc	387
Normal W Epi - unt	388
5	389
20	390
100	391
1000	392
Control 5	393
Control 1000	394
TCV1120 Control 5	395
Control 1000	396
1000 $\mu\text{g/ml}$ talc	397

383
384
385
OK 386

2/2/18

Prepare talc

100 mg in 10 mL \rightarrow mix and filter = 10 mg/mL

2/2/18 treated w/ talc

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(5 \text{ ug/mL}) = 2.5 \text{ mL}$$

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(20 \text{ ug/mL}) = 10 \text{ mL}$$

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(100 \text{ ug/mL}) = 50 \text{ mL}$$

2/6/18

treat w/ "soaked" talc (10000 ug/mL)

* talc was rocked for 72 hours, spun and supernatant collected

★ too much volume

made 1g/10 mL DMSO - re-soak 72 hrs

2/11/18 seeded Normal DU. Epi Cells $1 \times 10^6 \rightarrow$ treat Friday 2/8/18 ✓
2/26/18 Tuesday 2/21/18

383 NOE

0 ug/mL	383
5 ug/mL	384
20 ug/mL	385
100 ug/mL	386

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	356	2/5/2018 1:18:50 PM	0.0830	µg/µl	2.074	1.109	1.87	1.30	RNA	40.00
2	357	2/5/2018 1:19:20 PM	0.1000	µg/µl	2.500	1.342	1.86	1.18	RNA	40.00
3	358	2/5/2018 1:19:39 PM	0.0829	µg/µl	2.073	1.118	1.85	1.26	RNA	40.00
4	359	2/5/2018 1:20:00 PM	0.0349	µg/µl	0.873	0.476	1.84	0.39	RNA	40.00
5	360	2/5/2018 1:20:24 PM	0.2387	µg/µl	5.968	2.966	2.01	0.78	RNA	40.00
6	361	2/5/2018 1:20:43 PM	0.3389	µg/µl	8.473	4.194	2.02	1.15	RNA	40.00
7	362	2/5/2018 1:21:04 PM	0.3017	µg/µl	7.542	3.837	1.97	1.47	RNA	40.00
8	363	2/5/2018 1:21:20 PM	0.1118	µg/µl	2.796	1.465	1.91	1.53	RNA	40.00
9	368	2/5/2018 1:21:41 PM	0.2203	µg/µl	5.508	2.880	1.91	1.34	RNA	40.00
10	369	2/5/2018 1:21:57 PM	0.2474	µg/µl	6.185	3.187	1.94	2.03	RNA	40.00
11	370	2/5/2018 1:22:12 PM	0.2217	µg/µl	5.541	2.855	1.94	1.63	RNA	40.00
12	371	2/5/2018 1:22:29 PM	0.1336	µg/µl	3.340	1.726	1.93	1.42	RNA	40.00

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	379	2/16/2018 9:27:37 AM	0.1685	µg/µl	4.212	2.034	2.07	1.01	RNA	40.00
2	380	2/16/2018 9:27:55 AM	0.0658	µg/µl	1.645	0.713	2.31	3.02	RNA	40.00
3	381	2/16/2018 9:28:13 AM	0.0801	µg/µl	2.003	0.891	2.25	0.96	RNA	40.00
4	382	2/16/2018 9:28:30 AM	0.3084	µg/µl	7.711	3.759	2.05	2.24	RNA	40.00
5	383	2/16/2018 9:28:51 AM	0.2921	µg/µl	7.303	3.582	2.04	1.09	RNA	40.00
6	384	2/16/2018 9:29:10 AM	0.1812	µg/µl	4.531	2.179	2.08	2.15	RNA	40.00
7	385	2/16/2018 9:29:29 AM	0.0869	µg/µl	2.172	0.971	2.24	1.31	RNA	40.00
8	386	2/16/2018 9:29:51 AM	0.0116	µg/µl	0.289	-0.017	-16.61	-5.65	RNA	40.00
9	387	2/16/2018 9:30:11 AM	0.0133	µg/µl	0.332	0.013	25.53	-15.74	RNA	40.00
10	395	2/16/2018 9:30:29 AM	0.2169	µg/µl	5.421	2.598	2.09	0.65	RNA	40.00
11	397	2/16/2018 9:30:51 AM	0.1328	µg/µl	3.321	1.575	2.11	2.26	RNA	40.00
12	396	2/16/2018 9:31:08 AM	0.1633	µg/µl	4.084	1.949	2.10	2.47	RNA	40.00

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
16	364	2/16/2018 9:49:26 AM	0.2401	µg/µl	6.003	2.879	2.09	1.78	RNA	40.00
17	365	2/16/2018 9:49:46 AM	0.2418	µg/µl	6.044	2.939	2.06	1.27	RNA	40.00
18	366	2/16/2018 9:50:01 AM	0.2043	µg/µl	5.106	2.459	2.08	1.88	RNA	40.00
19	367	2/16/2018 9:50:16 AM	0.1712	µg/µl	4.281	2.026	2.11	1.83	RNA	40.00

QX
Super VILO
Kit

0.5 ug RNA Reaction

<u>ID</u>	<u>ul RNA</u>	<u>ul water</u>
356	6.0	10.0
357	5.0	11.0
358	6.0	10.0
359	14.3	1.7
360	2.1	13.9
361	1.5	14.5
362	1.7	14.3
363	4.5	11.5
364	2.1	13.9
365	2.1	13.9
366	2.4	13.6
367	2.9	13.1
368	2.3	13.7
369	2.0	14.0
370	2.3	13.7
371	3.7	12.3
379	3.0	13.0
380	7.6	8.4
381	6.2	9.8
382	1.6	14.4
383	1.7	14.3
384	2.8	13.2
385	5.8	10.2
386	16.0	0.0
387	16.0	0.0
395	2.3	13.7
397	3.8	12.2
396	3.1	12.9

